Remarks

1. Claim rejections under 35 USC 112, first paragraph

The patent office rejected claims 13-15, 21-25, and 28-31 based on the assertion that the specification did not enable those of skill in the art to practice the claimed invention. The Applicants traverse this rejection.

The enablement requirement of 35 USC 112 first paragraph requires a patent applicant to teach those of skill in the art to make and use the invention as claimed.

Claim 13 of the pending application, upon which all of the other pending claims depend, recites an automated method for analyzing distribution of a protein of interest between 2 cellular compartments, the cytoplasm and the cell membrane.

The specification provides a detailed example of a specific distribution assay, that between cytoplasm and nucleus (See, for example, Example 1 and Figure 6). The detailed discussion in the specification includes the following:

> Figure 6 is a representative display on a PC screen of data which was obtained in accordance with Example 1. Graph 1 300 plots the **difference between the average antibody fluorescence in the nuclear sampling region and cytoplasmic sampling region**, NucCyt Difference verses Well #. Graph 2 301 plots the **average fluorescence of the antibody in the nuclear sampling region, NP1 average,** versus the Well #. Graph 3 302 plots the **average antibody fluorescence in the cytoplasmic sampling region**, LIP1 average, versus Well #. The software permits displaying data from each cell. For example, Figure 5 shows a screen display 406, the nuclear image 401, and the fluorescent antibody image 402 for cell #14.
>
> **NucCyt Difference** referred to in graph 1 303 of Figure 6 is the **difference between the average cytoplasmic probe (fluorescent reporter molecule) intensity and the average nuclear probe (fluorescent reporter molecule) intensity**. The invention provides a **computer means for converting the digital signal from the camera into this parameter and for plotting the parameter** versus the well number.
>
> **NP1 average** referred to in graph 2 304 of Figure 6 is the average **of cyloplasmic probe (fluorescent reporter molecule) intensity within the nuclear sampling region. The invention provides a computer means for converting the digital signal from the camera into this parameter and for plotting the parameter** versus the well number.
>
> **L1P1 average** referred to in graph 3 305 of Figure 6 is the **average probe (fluorescent reporter molecule) intensity within the cytoplasmic sampling region. The invention provides a computer**

means for converting the digital signal from the camera into this parameter and for plotting the parameter verses the well number.

(Page 16 line 25 to page 17 line 15)

As can be seen, Example 1 and Figure 6 provide a detailed example of analyzing the distribution of a protein of interest between two cellular compartments (in this case, nucleus and cytoplasm) by a method comprising fluorescent reporter molecules reporting on the cell compartments of interest and the protein of interest (step (a) of the claim 13); automatically imaging cells to obtain the fluorescent signals from the reporter molecules and creating masks of the cell compartments of interest (step (b) of claim 13); automatically measuring the intensity of fluorescent signals from the reporter of the protein of interest within the masks of the cellular compartments of interest (step (c) of claim 13); and calculating a ratio or difference in intensity of the fluorescent signals from the reporter of the protein of interest between the masks of the different cellular compartments (step (d) of claim 13). Thus, Example 1 and Figure 6 clearly provide a detailed example of the general concept of translocation assays according to the invention, using as an example a translocation between cytoplasm and nucleus. The difference between the disclosure of Example 1 and Figure 6 and the present claims is the substitution of the cell membrane for the cell nucleus and the requirements for (a) using a fluorescent reporter of cell membranes; (b) creating a cell membrane mask from the fluorescent signals from the cell membrane reporter molecule; and (c) automatically measuring the intensity of fluorescent signals from the reporter of the protein of interest within the cell membrane mask (and comparing it as recited in the claims to signals from the reporter of the protein of interest in the cytoplasmic mask, which the Patent Office has acknowledged is enabled).

The Patent Office does not assert that those of skill in the art would not be able to identify appropriate markers of cell membranes that can be fluorescently labeled and used in the present invention. Such markers and their use to label cell membranes were well known to those of skill in the art at the time the application was filed. Similarly, the specification teaches creating masks of cellular compartments (nucleus and cytoplasm) from the fluorescent signals of the cytoplasmic and nuclear reporters; those of skill in the art would

certainly be able to use the teachings of the present invention as well as the general knowledge in the art to create masks from fluorescent signals of membrane reporters.

Furthermore, the present application provides explicit teaching that the methods disclosed in the invention can be used to analyze translocation events between different cellular compartments. In describing the basis for translocation assays between the cytoplasm and nucleus, the Applicants stated:

> Features 5-9 have been developed specifically to provide measurements of a cell's fluorescent molecules within the local cytoplasmic region of the cell and the translocation (i.e. movement) of fluorescent molecules from the cytoplasm to the nucleus. These screen specific features are used for analyzing cells in microplates for the inhibition of nuclear translocation. Inhibition of nuclear translocation of transcription factors provides a novel approach to screening intact cells. An automated screen of an inhibitor of NF-κB translocation has been successfully performed. A specific algorithm measures the amount of NF-κB probe in the nuclear region (feature 4) versus the local cytoplasmic region (feature 7) of each cell. **Quantification of the difference between these two sub-cellular compartments provides a measure of cytoplasm-nuclear translocation (feature 9).**
>
> (Page 12 lines 23-31)

This statement clearly contemplates that quantification of the same difference between **other sub-cellular compartments** would provide a measure of distribution between those other sub-cellular compartments. This is further confirmed by the text on Page 19 lines 3-13:

> **Those skilled in the art will recognize a wide variety of distinct screens that can be developed**. There is a large and growing list of known biochemical and molecular processes in cells that involve translocations or reorganizations of specific components within cells. The signaling pathway from the cell surface to target sites within the cell involves the **translocation of plasma membrane-associated proteins to the cytoplasm**. For example, it is known that one of the src family of protein tyrosine kinases, pp60c-src, **translocates from the plasma membrane to the cytoplasm** upon stimulation of fibroblasts with platelet-derived growth factor (PDGF). In contrast, some cytoplasmic components translocate from the cytoplasm to the plasma membrane upon stimulation of cells. For example, it is known that the GTP-binding proteins of the Rho family are **maintained as cytoplasmic complexes with RhoGDI in resting cells, but are released and translocate to plasma membrane** during cell activation.

Therefore, the Applicants have taught those of skill in the art that, using the methods disclosed in the application and exemplified in the Example cytoplasm-nucleus assay, other sub-cellular distribution assays, such as cytoplasm-cell membrane, can be carried out. As a result, the specification provides enablement for those of skill in the art to practice the claimed invention.

The applicants therefore respectfully request reconsideration and withdrawal of the rejection.

Based on all of the above, the applicants believe that the application is in condition for allowance. If the Examiner believes that a telephone or personal interview would expedite prosecution of the instant application, the Examiner is invited to call the undersigned attorney at (312) 913-2106.

Respectfully submitted,

McDonnell Boehnen Hulbert & Berghoff

Date: 4/1/05

By: _____

David S. Harper
Registration No. 42,636